Reduction of furan formation by high pressure-high temperature treatment of individual vegetable purées

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Reduction of furan formation by HPHT treatment

Abstract

The present study addressed the need for furan mitigation measures at the level of food production, where the effects of extrinsic (process-related) and intrinsic (product-related) properties on furan formation in vegetable-based systems were investigated. For the first time in the literature, the effect of high pressure-high temperature (HPHT) processing on the formation of furan was demonstrated. HPHT processing was proven to be an interesting alternative for furan reduction in vegetable-based systems, when aiming for sterilization intensities. Following HPHT treatment, the furan concentrations of a wide range of individual vegetable purées dropped to levels close to the analytical limits (1-2 ng/g purée). A higher processing cost might limit the use of HPHT processing to high-value added products, which means that for many other conduction-heated food products, conventional heating would remain the standard technology. As a first step towards control of furan formation in the latter products, a mixed model regression was used to identify the major precursors in vegetable-based systems. Significant

correlations were observed for vitamin C and sugars, which were attributed to the efficiency of the conversion and high concentrations, respectively. Next to furan, the HPHT- and thermally-treated purées were analyzed for 2- and 3-methylfuran, which are likely to undergo the same metabolic fate as furan. For most of the vegetables tested, the total amount of methylfuran found in the thermally-treated purées could not be ignored. Similarly to furan, there was a clear reduction in the concentrations found in the HPHT-treated purées.

Keywords

Furan; methylfuran; precursor; vegetable; thermal processing; high pressure-high temperature processing.

1. Introduction

Furan (C_4H_4O) is a small organic molecule with high volatility. Historically, furan and derivatives were recognized as contributors to the sensory properties of a wide variety of foods (Maga, 1979). However, in 1995, furan was classified as 'possibly carcinogenic' to humans after it was proven to be carcinogenic in rats and mice (International Agency for Research on Cancer (IARC), 1995). By 2006, American as well as European food safety authorities had published requests for more data on the occurrence of furan in foods, on the mechanisms of formation and on the toxicological effects. A recent report published by the European Food Safety Authority (EFSA) (2011) gives an update on the furan levels in foods from monitoring years 2004-2010 and an exposure assessment for European consumers. Exposure estimates are the highest in toddlers (0.05-0.31 $\mu g/kg_{bw}$/day) and in adults (0.03-0.59 $\mu g/kg_{bw}$/day) with jarred baby foods and coffee being the major contributors, respectively. The highest furan concentrations are found in food products which have undergone an extensive heat treatment such as roasting or sterilization. The latter partly explains why low-acid, conduction-heated foods (often vegetable-based systems like jarred baby foods, ready-to-eat soups, sauces and juices) are particularly susceptible to the formation of furan. In the literature, many ways leading to the formation of furan have

been reported, the major precursors being sugars (alone or in combination with amino acids), ascorbic acid and unsaturated fatty acids, followed by amino acids and carotenoids (Locas & Yaylayan, 2004; Becalski & Seaman, 2005; Fan, 2005; Mark *et al*., 2006; Limacher *et al*., 2007; Limacher *et al*., 2008; Owczarek-Fendor *et al*., 2010a; Owczarek-Fendor *et al*., 2011; Van Lancker *et al*., 2011; Huang *et al*., 2011; Owczarek-Fendor *et al*., 2012). Interactions between these precursors and reaction conditions linked to the composition of the product (pH, redox condition) can have a great effect on the amount of furan formed in the product (Fan *et al*., 2008; Van Lancker *et al*., 2009; Owczarek-Fendor *et al*., 2010a; Owczarek-Fendor *et al*., 2010b; Owczarek-Fendor *et al*., 2012).

Given the toxicological properties of furan and the outcome of the risk characterization, actions should be taken to minimize exposure to an acceptable level. Research has been done on ways to reduce the furan concentrations in heated foods (Crews & Castle, 2007; Blank, 2009; Anese *et al*., 2013), but this has not yet resulted in clear solutions, as the exact mechanisms for its formation are not fully understood (Stadler & Lineback, 2008). Basically, there are three possible approaches for the mitigation of furan: (i) changing the composition (and reaction pathways) by adding or removing substances from the product, (ii) optimization of the conventional heating process or application of an alternative processing technique (e.g. high pressure processing) and (iii) post-process reduction of the amount of furan formed (e.g. ionizing radiation, vacuum treatment). On the food composition side, the best approach to reduce furan formation appears to be intervention in the reaction mechanisms (Crews & Castle, 2007). Furan has a wide range of precursors and it is not desirable to remove them from the matrix because of their structural properties and health benefits. With regard to process conditions, microbial safety standards may limit the scope for lowering heating times and temperatures, e.g. for the in-pack sterilization of conduction-heated food products. Furthermore, furan and its derivatives are known to be important end-products of the Maillard reactions in foods (e.g. coffee). As a result, measures to reduce furan formation might result in processed products with a different and unacceptable flavor profile for the consumer.

High pressure processing is one of the alternative processing techniques that has been given a lot of research attention in the recent search for foods with high quality properties. If aimed at shelf-stable low-acid products, high pressure (typically around 600 MPa) has to be combined with high temperature (typically 90 to 130 °C), since bacterial spores are highly pressure resistant (Mujica-Paz *et al*., 2011). The application of the process parameter pressure in such a combined high pressure-high temperature (HPHT) treatment allows faster heating and cooling rates, which can result in shorter processing times compared with a conventional thermal treatment (Barbosa-Canovas & Juliano, 2008). Furthermore, both pressure and temperature have an effect on the rate constants of microbiological inactivation as well as quality-related chemical reactions (Heinz & Knorr, 2005). For low acid, conduction-heated food products, the integrated effect of pressure, temperature and time can result in a better preservation of the original quality attributes (Barbosa-Canovas & Rodriguez, 2005; Rastogi *et al*., 2007). However, because of the specific effect of the process parameter pressure on the formation reactions, it is difficult to predict whether HPHT processing will have a positive, neutral or negative effect on the level of undesirable substances, such as the furan concentration in foods.

In the past, little attention has been given to the fact that in most heated foods, furan is accompanied by a series of alkylated derivatives (Maga, 1979). At least two of them, namely 2-methylfuran and 3-methylfuran, might be of toxicological interest, since animal studies have shown that they are metabolically activated in a similar way as furan, resulting in hepato- and pulmonary toxicity (Wiley *et al*., 1984; Gammal *et al*., 1984; Ravindranath & Boyd, 1985; Ravindranath *et al*., 1986). However, it is possible that the formation of both compounds in heat-treated foods is influenced in its own way, different from furan, as their formation seems to be more affected by the recombination of fragments coming from sugars and amino acids. The mechanisms of formation need more elucidation, but also ascorbic acid, unsaturated fatty acids and β-carotene have been reported as a precursor for the formation of methylfuran (Mark *et al*., 2006; Limacher *et al*., 2007; Limacher *et al*., 2008). Given all this information, it might be appropriate to also take into account the concentrations of these alkylated derivatives when performing a risk evaluation of furan, as recently proposed by Becalski *et al.* (2010)

and as adopted in the recommendations of the Joint FAO/WHO Codex Alimentarius Commission (2011).

The main objective of the present study is to obtain a better understanding of the furan formation in vegetable-based food products. As apparent from the recent EFSA report (2011), thermally processed, vegetable-based foods are important contributors to furan exposure for both infants and adults. Understanding furan formation in vegetable-based food products will help to identify opportunities for furan reduction. For the first time in the literature, the effect of HPHT processing on the formation of furan will be demonstrated. For a fair comparison of HPHT with thermal processing, the vegetable-based systems selected in this study will be treated to provide an equivalent microbial impact. As a first step towards control of furan formation in conventionally, thermally-treated products, the major furan precursors in vegetable-based systems will be identified using a mixed model regression. Furthermore, the formation of 2- and 3-methylfuran will be monitored, following the recommendations of the Joint FAO/WHO Codex Alimentarius Commission (2011). In a similar approach to that for furan, both the effects of HPHT processing and chemical composition on the total amount of methylfuran will be investigated.

2. Materials and methods

2.1. Sample preparation

A well-considered selection of fresh vegetables (*cf.* Section 3.1) was bought at a local shop. The selection included bell pepper, broccoli, four different color varieties of carrot, onion, potato, pumpkin, red beet and spinach. All vegetables were carefully washed and cut into standardized shapes. All carrots, potato, pumpkin and red beet were cut into small cylinders of approximately 2 cm diameter and 1 cm thickness. For bell pepper, the seeds and petiole were discarded and the flesh was cut into strips approximately 0.5-1 cm wide. Broccoli was cut into florets of 2.5 cm with 1 cm stem and onions into small pieces of 1 cm. For spinach, only the petiole was removed. The vegetable pieces were vacuum packed in low density polyethylene bags. To assure that all the changes observed during thermal and

143 HPHT processing were chemical, the vegetables were blanched at 95 °C for 8 min in a water bath
144 (WBU 45, Memmert, Germany). The blanching conditions were validated using a qualitative and
145 quantitative peroxidase test (Adebooye *et al*., 2008; Vervoort *et al*., 2012). After blanching, the plastic
146 bags were immediately cooled in iced water for 10 minutes, frozen in liquid nitrogen and stored in a
147 freezer at –40 °C. Prior to thermal or HPHT treatment, the vegetable pieces were thawed overnight in a
148 cold room at 4 °C and blended (B-400, BÜCHI, Switzerland) with a standardized amount of deionized
149 water to obtain homogeneous individual vegetable purées.
150
151 *2.2. Processing*
152 To obtain a fair comparison for the process impact (Vervoort *et al*., 2011), an equivalent industrially
153 relevant process value $F_{121.1\,^{\circ}C}^{10\,^{\circ}C}$ (F_0) = 5 min was proposed for both thermal and HPHT processing,
154 targeting the equivalent inactivation of *Clostridium botulinum* spores. A holding temperature (T_h) of 117
155 °C was selected for both treatments. Each treatment was repeated six times. Due to the lack of reliable
156 kinetic data, as a result of an incomplete understanding of the combined effect of pressure and
157 temperature on *Clostridium botulinum* spore inactivation (Van der Plancken *et al*., 2012), in the present
158 study the HPHT treatment was considered as pressure-assisted thermal processing. In other words, a
159 complete lack of synergy between pressure and temperature was assumed, and HPHT sterilization
160 conditions were chosen to be at least thermally equivalent to the thermal treatment. Due to their inert
161 nature, glass jars were selected for the thermal treatment and Teflon (polytetrafluoroethylene) sample
162 holders for the HPHT treatment.
163
164 *2.2.1. Thermal treatment*
165 The thermal treatment was carried out in a static Steriflow pilot retort (Barriquand, France). The glass
166 jars (100 ml volume, 95 mm height and 45 mm diameter) were filled with 85 ± 0.5 g of vegetable purée
167 and then closed with metal lids. Next, they were loaded into the retort and sterilized during processing

168 times of 80 min. Temperature profiles in the retort and at the coldest point of the product were recorded
169 using type T thermocouples (Ellab, Denmark) (**Fig. 1**, bold dark line).
170
171 *2.2.2. High pressure-high temperature treatment*
172 The HPHT treatment was carried out in laboratory-scale HPHT equipment (custom-made, Resato,
173 The Netherlands). The equipment consists of six vertically oriented individual vessels (43 cm^3 volume
174 and 2 cm diameter). The vessels were jacketed with a heating coil connected to a temperature
175 controlling unit. The HPHT equipment allows computer-controlled pressure build-up to 800 MPa,
176 temperature control up to 120 °C and data logging of both sample pressure (**Fig. 1**, dashed light line)
177 and temperature (**Fig. 1**, bold light line). The pressure medium was 100% propylene glycol (PG fluid,
178 Resato, The Netherlands). During HPHT treatment, a preheating step at atmospheric pressure, pressure
179 build-up, a holding and a cooling step should be considered (Grauwet *et al*., 2010; Grauwet *et al*., 2012;
180 Van der Plancken *et al*., 2012). In this work, the Teflon sample holders (12 mm inner diameter, 85 mm
181 length and 4 mm wall thickness, Vink, Belgium) were filled with vegetable purée, closed with a
182 movable cap and vacuum-sealed with double plastic bags. The sample holders were pre-equilibrated at
183 10 °C in a cryostat and loaded into the HP vessels that were equilibrated at the holding temperature of
184 117 °C. Starting from room temperature, using only compression heating, the product temperature
185 cannot be raised to the point where inactivation of spores under high pressure is feasible. Therefore,
186 before the actual HPHT treatment, the samples were preheated at atmospheric pressure to an
187 experimentally determined initial temperature (T_i) of 75 °C. When the desired T_i was achieved, the
188 pressure in the vessels was increased by pumping the pressure medium in the vessels (through indirect
189 compression). During the pressure build-up by the intensifier, two consecutive stages can be identified:
190 (i) an instaneous pressure increase from 0.1 to 150 MPa; (ii) a further pressure increase to 600 MPa at a
191 rate of 10 MPa/s. After reaching 600 MPa, the individual vessels were isolated and an equilibration time
192 of 1 min was taken into account. Due to the pressurization and isolation phases, the temperature inside
193 the product increased from T_i to 117 °C through compression heating. The product temperature was

recorded online and the holding time was corrected to achieve the target F_0 value. On average, the pressure was held for 15 min. At the end of the holding time, the pressure was released from the vessels, which was accompanied with a temperature drop inside the product (decompression cooling).

2.3. Post processing sample handling

Following treatments, samples were immediately transferred to iced water to stop any further reaction. Consequently, treated samples were emptied in a cold room at 4 °C and transferred to small volume (10 ml) polyethylene terephtalate tubes with a polethylene cap. Hereafter, the tubes were frozen in liquid nitrogen and stored at –40 °C until analysis.

2.4. Analytical procedures

2.4.1. Quantification of furan and methylfuran

Furan was analyzed with solid phase microextraction coupled to gas chromatography-mass spectrometry (SPME-GC-MS), using furan-d_4 as an internal standard. Because of the high volatility of furan, precautions had to be taken to obtain reliable and reproducible results. Standard stock solutions (ca. 2.5 mg/ml in methanol) and working solutions (ca. 0.25 µg/ml in deionized water) were obtained by making a serial dilution of furan (≥ 99%, Sigma Aldrich, USA) in closed headspace vials of 10 ml. Furan was added to the solvent with a chilled gastight syringe through the septum of the vials. Both the amount of solvent and the amount of solution added were determined by differential weighing and the furan concentration was calculated from the respective masses. An analogous approach was followed for the preparation of the furan-d_4 (98%, Sigma Aldrich, USA) standard stock solutions and working solutions. All operations were done in a closed and refrigerated sample preparation box (MPR-311D(H), Sanyo, Japan). The solutions were prepared daily and stored on ice.

The samples were prepared for analysis by weighing 2.5 g of the cooled purée in an empty 10 ml headspace vial with a PTFE/silicone septum seal. Before the vial was completely closed, 2.5 ml of a saturated NaCl solution was added to the purée and the mixture was further diluted with deionized water

220 to obtain a standardized total volume of 6 ml. After sealing, 100 µl of the internal standard working
221 solution was added with a chilled gastight syringe. The exact amounts were determined by differential
222 weighing. The samples were stored at a temperature of 10 °C until analysis.

223 The analyses were carried out using an Agilent 7890A GC and an Agilent 5975C MS (Agilent
224 Technologies, USA), equipped with a CTC Combi PAL autosampler (CTC Analytics, Switzerland). The
225 SPME fiber (Supelco, USA) had a 85 µm carboxen/polydimethylsiloxane (CAR/PDMS) sorptive
226 coating, which was exposed to the headspace of the samples for 15 min at 30 °C. After headspace
227 extraction, the fiber was transferred to the GC injection port, where the adsorbed compounds were
228 thermally desorbed for 1 min at 200 °C. After each run, the fiber was thermally cleaned for 2 min at 300
229 °C in the conditioning station of the autosampler. The volatiles were injected in the splitless mode and
230 subsequently separated on a HP-PLOT Q column (30 m × 320 µm, 20 µm film thickness, Agilent
231 Technologies, USA), using helium as the carrier gas at a constant flow rate of 2 ml/min. The column
232 oven was programmed at a starting temperature of 40 °C, which was retained for 4 min, after which it
233 was elevated to 160 °C at a rate of 40 °C/min, followed by a second ramp to 220 °C at 5 °C/min. After 1
234 min at the final temperature, the oven was cooled again to the initial temperature. Mass spectra were
235 obtained by electron ionisation (EI) at 200 eV, in the combined SCAN and SIM mode. The scanning
236 range extended m/z 35-400. The selected ions monitored were m/z 68 (quantifier) and 39 (qualifier) for
237 furan and m/z 72 (quantifier), 44 and 42 (both qualifier) for furan-d_4. MS ion source and quadrupole
238 temperatures were 230 and 150 °C respectively.

239 The method of internal standard calibration was used to prepare a calibration curve for furan. A serial
240 dilution was made starting from the furan working standard solution, resulting in a calibration curve
241 covering the concentration range of 0-50 ng/g purée. The identity of both furan and furan-d_4 was
242 confirmed by calculating the response ratio of the qualifier ions and the quantifier ions according to the
243 guidelines stated in European Commission Decision (2002/657/EC).

244 The method was validated in terms of specificity, recovery, precision (repeatability), decision limit
245 (CCα) and detection capability (CCβ), using the above-mentioned European Commission Decision

246 (2002/657/EC) concerning the performance of analytical methods and the interpretation of results. The
247 specificity of the method was demonstrated by analyzing different blank and treated samples and from
248 spiking experiments. In all cases no interferences were observed. The concentration levels selected for
249 spiking were 1, 10 and 50 ng/g purée and covered the entire range of furan concentrations detected. The
250 spiking experiment was repeated in four different matrices (milli-Q water, bell pepper, broccoli and
251 spinach) and each concentration level was prepared in sixfold. The results showed good recoveries,
252 varying between 95 and 120%, depending on the spiking level. The repeatability was determined at the
253 same concentration levels and demonstrated the precision of the procedure, resulting in RSDs between
254 1.03 and 12.81%. The decision limit and the detection capability were 1.15 ng/g purée and 1.86 ng/g
255 purée respectively. Both parameters were established by the calibration curve procedure.
256 With some small modifications, the analytical procedure for furan as described above could also be
257 used to estimate the concentrations of 2-methylfuran and 3-methylfuran in the samples (Limacher *et al*.,
258 2007; Limacher *et al*., 2008). According to our own findings (results not shown), the behaviour of both
259 methylfurans towards the internal standard furan-d_4 was not significantly different from the behaviour
260 of furan. Therefore, 2-methylfuran and 3-methylfuran were quantified with the same calibration curve
261 as used for furan. For both compounds, the selected ions monitored were m/z 82 (quantifier), 81 and 53
262 (qualifier). The identity of both 2-methylfuran and 3-methylfuran was confirmed by comparison with
263 retention times of standards and by calculating the response ratio of the qualifier ions and the quantifier
264 ions, as analogous to the identification of furan and furan-d_4.
265
266 *2.4.2. Determination of the free sugar content*
267 Free sugars were determined by reversed-phase high-performance liquid chromatography (RP-HPLC)
268 with evaporative light scattering detection (ELSD), according to the procedure of Vervoort *et al.* (2012).
269 The extraction procedure was based on the method of O'Donoghue (2004), with small modifications.
270 2.5 g of vegetable purée was combined with 5 ml of 62.5% (v/v) methanol and mixed. The resulting
271 slurry was placed in a shaking water bath at 55 °C for 15 min, including vortex mixing for 20 s every 5

min. The samples were cooled on ice and afterwards centrifuged for 10 min at 16000 × g and 4 °C (J2-HS Centrifuge, Beckman Coulter, Belgium). The supernatant was filtered through a 0.45 µm syringe filter and 15 µl of the filtrate was injected into the HPLC system. Each vegetable purée was extracted in triplicate.

The HPLC system consisted of the Agilent 1200 Series apparatus (Agilent Technologies, Belgium) equipped with an external Alltech 3300 ELSD detector (Grace, USA). For all HPLC analyses, the autosampler was cooled to 4 °C. Sugars were separated on an Alltech Prevail Carbohydrate ES column (250 × 4.6 mm, 5 µm particle size, Grace, USA), by isocratic elution using 75% (v/v) acetonitrile/water at a flow rate of 1 ml/min and 30 °C. The drift tube temperature for ELSD was set at 38 °C and nitrogen was used as the nebulizer gas at a flow rate of 1.5 ml/min. Standard solutions of different sugars were prepared in 62.5% (v/v) methanol. Quantification and identification of the sugars was performed by setting up calibration curves based on the peak areas of the injected standards and by comparison of their retention times, respectively. Each sample extract was analyzed once. All solvents used for HPLC analyses were HPLC-grade.

2.4.3. Determination of the vitamin C content

Vitamin C consists of two fractions: ascorbic acid (AA) and dehydroascorbic acid (DHAA). The concentrations of both fractions were determined by RP-HPLC with UV detection, according to the procedure of Verbeyst *et al.* (2013). Vitamin C was extracted from the vegetable purées by adding extraction buffer (1% (w/v) m-phosphoric acid + 0.5% (w/v) oxalic acid in milli-Q water, adjusted to pH 2.0) in an optimized ratio of 1:2. After mixing for 1 min, this mixture was centrifuged for 30 min at 20400 × g and 4 °C (J2-HS Centrifuge, Beckman Coulter, Belgium) and filtered through a 0.45 µm syringe filter. Each sample was extracted in duplicate.

To determine the vitamin C content, extracts were adjusted to pH 3.5 and divided into two parts. The first part was used for direct analysis of AA. After adding phosphate buffer (20 mM NaH_2PO_4 + 1 mM

297 Na_2EDTA in milli-Q water, adjusted to pH 3.5) in a ratio of 1:2, this sample was ready for HPLC
298 analysis. The second part was subjected to a reduction reaction of DHAA to AA by adding TCEP
299 solution (2.5 mM tris (2-carboxyl-ethyl) phosphine in phosphate buffer, adjusted to pH 3.5) in a ratio of
300 1:2 and centrifuging for 15 min at 19000 × g and 24 °C (Microfuge 22R, Beckman Coulter, Belgium).
301 This way, all DHAA was converted to AA, so that in the HPLC analysis, the total vitamin C content
302 was measured in the form of AA. By subtracting the AA concentration measured in the first part of the
303 extract from the concentration measured in the residual part, the DHAA concentration was calculated.
304 HPLC analysis was carried out with a Dionex BioLC apparatus, using isocratic elution over a Prevail
305 C18 column (250 × 4.6 mm, 5 µm particle diameter, Grace, USA) held at 25 °C. The elution buffer (1
306 mM Na_2EDTA + 10 mM CH_3COONH_4 in milli-Q water, adjusted to pH 3.0) had a flow rate of 0.8
307 ml/min. The injection volume (25, 50 or 100 µl) was adjusted depending on the expected amount of AA
308 in the sample and the absorbance was measured at 245 nm. A standard curve of AA (99%, Acros
309 Organics, Belgium) was prepared in extraction buffer. Quantification and identification of AA were
310 done by setting up a calibration curve based on the peak area of the injected standards and by
311 comparison with their retention time, respectively. The exact concentration of the standard solution was
312 determined spectrophotometrically (245 nm, 25 °C, pH 0.69, $\varepsilon = 10.2\ mM^{-1}cm^{-1}$) according to the law
313 of Lambert-Beer. Each extract was analyzed once for AA and total vitamin C.
314
315 *2.5. Statistical data analysis*
316 Statistical data analysis was performed using the SAS statistical software package (SAS Enterprise
317 Guide 4.3, USA). A mixed model was applied, with fixed factors to estimate the effects of the
318 controllable factors and random factors to correct for the correlation between samples coming from the
319 same vegetable. Significant differences among the fixed factors were examined using the post-hoc
320 Tukey test at a significance level of 0.05.
321

3. Results and discussion

3.1. Selection of the vegetables

A series of vegetables was selected, aiming for a wide range of natural compositions and to represent natural mixtures of all known furan precursors. A first selection of vegetables was set up based on differences in botanic family, plant part and colour. According to Pennington and Fisher (2009), these selection criteria could be correlated with the presence and particular levels of food components in fruit and vegetables. The first group of vegetables included broccoli, onion, potato, pumpkin and red beet. However in the context of furan formation, vitamin C and carotenoids were found to be underrepresented in this group compared with other known precursors such as sugars and unsaturated fatty acids. Therefore, green bell pepper and spinach were added as rich sources of vitamin C and orange-, purple-, red- and yellow-coloured carrots were added as important sources of carotenoids. An overview of the selected vegetables and their cultivar/variety are given in **Table 1**.

3.2. Effect of different sterilization techniques on the furan formation in vegetable purées

Extrinsic properties like the intensity of the process have an important contribution to the amount of furan formed in the product, as also reflected from the recent EFSA report (2011). The present study has focused on the effects of conventional in-pack thermal processing and innovative HPHT processing on furan formation in vegetable-based products, by comparing the integrated impact of both sterilization techniques. For a fair comparison of the treatments, all the vegetable purées selected in this study were treated by aiming an equivalent microbial impact (F_0 = 5 min).

3.2.1. General observations

The furan concentrations found in the thermally- and HPHT-treated purées are represented in **Fig. 2**. Several conclusions could be drawn from this comparison. A very interesting finding was the clearly different and lower concentrations of furan found in the vegetable purées after HPHT treatment. The furan concentrations dropped to levels close to the limits of the analytical procedure (1-2 ng/g purée),

348 which means that almost no furan was detected in the HPHT-treated purées, even though they were
349 treated under industrially relevant conditions for sterilization. Moreover, it should be noted that a
350 reduction in furan concentration could be observed for the entire series of vegetables selected for this
351 study. Therefore, HPHT processing presents itself as a really interesting alternative for furan reduction
352 in sterilized vegetable-based products.

353 The higher furan concentrations observed for the thermally-treated purées were characterized by a
354 larger variability among the vegetables compared with the HPHT-treated purées. Red beet had the
355 highest concentration (15 ng/g purée), followed by bell pepper, spinach and onion. Pumpkin, potato and
356 red carrot had average concentrations (7-8 ng/g purée). Broccoli and the other carrot varieties (2-4 ng/g
357 purée) seemed to be the least susceptible to furan formation. Information on the susceptibility of pure
358 vegetables to furan formation could help to identify risk matrices and to reformulate products in order to
359 reduce furan concentrations.

360

361 *3.2.2. Mixed model regression*

362 In order to obtain statistical insight into the significance of the trends described above, a mixed model
363 regression was used to mathematically describe furan formation in the treated vegetable purées. The
364 definition of this model, built with one fixed factor (treatment type) and one random factor (vegetable
365 type), is given in the following equation:

366

367 $$Y_{ij} = \mu + b_i + \beta x_j + \varepsilon \qquad (1)$$

368

369 Where Y is the furan concentration, μ the intercept, b the effect of vegetable type and β the effect of
370 treatment type, for the i^{th} vegetable purée and the j^{th} repetition of the treatment. The results of the
371 statistical analysis for the fixed and random effects are given in **Table 2**.

372

In this mixed model, the intercept μ corresponds to the average furan concentration in the thermally-treated vegetable purées and the fixed effect β to the average reduction for the HPHT-treated purées. Both effects were shown to be significant (**Table 2**, fixed effects). In terms of percentages, which can be calculated from **Fig. 2**, the average reduction by HPHT treatment compared with conventional thermal sterilization amounted to 76%, with a minimum of 46% (yellow carrot) and a maximum of 91% (pumpkin). Two possible reasons for the reduction of the furan concentrations in the HPHT-treated purées can be proposed. On the one hand, high pressure can have a different effect on the rate constants of chemical reactions. In this context, high pressure could have slowed down furan formation. Consulting the literature on the knowledge of the effect of high pressure on furan formation, no information could be found. However, some information was available about the effect of high pressure on the degradation reactions of particular furan precursors. High pressure may have a slowing-down effect on the overall Maillard reaction (De Vleeschouwer *et al*., 2010), but an enhancing effect on the oxidative thermal degradation of ascorbic acid and unsaturated fatty acids (Verbeyst *et al*., 2013; Kebede *et al*., 2013). Carotenoids do not seem to be clearly affected by high pressure (Knockaert *et al*., 2011). Consequently, both slowing-down and enhancing effects of high pressure are described, making it difficult to speculate on the concerted effect of the furan forming reactions. On the other hand, the temperature-time profiles of both treatments were clearly different (**Fig. 1**). Even in the case where no effect of pressure on the reactions is assumed and the particular effect of temperature on the reactions is taken into account (as in the experimental design of this study), the clearly shorter process time in the context of HPHT processing could be the reason for the distinctly lower furan concentrations detected in these purées. Of course, the trends observed could also be due to a combination of both reasons addressed above. This study aimed to compare the integrated impact of the two sterilization techniques, resulting in relevant concentrations and conclusions from the application point-of-view. To obtain a clear insight into the individual effect of the processing variables, a kinetic study needs to be set up.

The mixed model discussed above (Eq. 1) could also be used to classify the vegetable purées with regard to their susceptibility to furan formation, by comparing the estimated random effects b_i (**Table 2**, random effects). The values can be interpreted as an individual correction of the furan concentration for each vegetable type, taking into account the final concentrations of both the thermal and the HPHT treatment. In other words, a positive value stands for a higher than average furan concentration, irrespective of the treatment type, and vice versa. A comparison of these random effects confirmed that red beet was the vegetable most susceptible to furan formation, followed by bell pepper and spinach. Broccoli and the carrots seemed to be less susceptible to furan formation, compared with the other vegetables selected for this study. These results agreed with those from a recent food survey conducted by Health Canada (Becalski *et al*., 2010), where beets were found to contain the highest furan concentrations among different canned and jarred vegetables. Also, the rankings of potatoes and carrots were the same as our rankings. The classification of the vegetables with regard to their susceptibility to furan formation identified red beet, bell pepper and spinach as possible risk matrices for furan formation. In particular, spinach is a frequently used ingredient in vegetable-based products like jarred baby foods. If possible, the combination of such ingredients should be limited in order to avoid the formation of high amounts of furan in the product. Much research still has to be done in this area, since it is clear that in real systems, reaction conditions and interactions play an important role in the amount of furan that is formed.

3.3. Effect of chemical composition on the furan formation in thermally-treated vegetable purées

Although this work has proved that HPHT processing shows great potential in mitigating furan concentrations in conduction-heated vegetable-based food products, a lot of scientific, technical and legislative issues are still to be overcome before HPHT processing can serve as a real sterilization alternative (Rastogi *et al*., 2007; Balasubramaniam & Farkas, 2008). Furthermore, the equipment and operating costs might limit the use of HPHT processing only to high-value added foods (Bermudez-

423 Aguirre & Barbosa-Canovas, 2011; Mujica-Paz *et al*., 2011). Therefore, it is important that research on
424 furan reduction in conventionally thermally-treated foods is not abandoned.
425
426 *3.3.1. Free sugars and vitamin C as major furan precursors*
427 As a first step towards control, there is a need for insight into the intrinsic mechanisms of furan
428 formation during thermal treatment. In this context, carbohydrates, ascorbic acid and unsaturated fatty
429 acids are named as important furan precursors (Fan, 2005; Crews & Castle, 2007; Moro *et al*., 2012).
430 Since Owczarek-Fendor *et al.* (2010b) observed furan to be mainly formed at unrealistically high levels
431 of fatty acid oxidation, the present study focused on the importance of carbohydrates and vitamin C in
432 forming furan in conduction-heated vegetable-based food products. As a start, the concentrations of
433 both precursors were determined in the starting material (blanched vegetable purées) and related to the
434 concentration of furan in the end products (treated purées) by means of a mixed model regression.
435
436 *3.3.1.1. Characterization of the starting material*
437 The free sugar content of the samples was determined as a measure of the furan forming potential of
438 carbohydrates. Some literature sources (Owczarek-Fendor *et al*., 2010a) suggest that furan can also be
439 formed from starch, but the amounts are expected to be small, since the individual glucose units first
440 have to be released in order to be degraded to furan. The results of the free sugar analyses are
441 represented in **Fig. 3**. Fructose, glucose and sucrose were the only free sugars detected in quantifiable
442 amounts. Red carrot had the highest concentration of free sugars (1.6 g/100 g purée), followed by
443 pumpkin, red beet, onion and the other carrots. Compared with these vegetables, the amounts of free
444 sugars in potato and spinach were very low (0.2-0.3 g/100 g purée). For onion and the carrots, the
445 amounts of fructose, glucose and sucrose were very comparable to each other, whereas the free sugars
446 of pumpkin and red beet mainly consisted of sucrose.
447 The vitamin C content of the blanched vegetable purées is represented in **Fig. 4**. Bell pepper (129
448 mg/100 g purée), broccoli (51 mg/100 g purée) and spinach (30 mg/100 g purée) had a vitamin C

449 content that was considerably higher than the other vegetables (2-12 mg/100 g purée). In all cases,
450 vitamin C mostly consisted of AA, except for spinach, which could be explained by its leafy nature.
451
452 *3.3.1.2. Mixed model regression*
453 The furan concentrations of the thermally-treated vegetable purées are already shown in **Fig. 2**.
454 Visually, it was almost impossible to detect any associations between the chemical composition of the
455 starting material and the furan concentrations of the thermally-treated purées. Mixed model regression
456 was a helpful tool to obtain statistical insight into the significance of the effect of chemical composition,
457 even more than in the case of the effect of different sterilization techniques. By analyzing the remaining
458 vitamin C concentrations of the thermally-treated purées, the precursor could be included in the model
459 as the amount of degraded vitamin C, which was decided to be a better indicator for the furan forming
460 potential of vitamin C than the initial concentration. In most of the cases (not for bell pepper, broccoli
461 and spinach), the initial amount of vitamin C was nearly completely degraded during the treatment
462 (results not shown). The same approach was not possible for the amount of free sugars, since small
463 amounts of free sugars could be formed due to partial hydrolysis of polysaccharides during the
464 treatment. The final mixed model consisted of four fixed factors (initial concentration of fructose,
465 glucose and sucrose and amount of degraded vitamin C) and one random factor (vegetable type), as
466 represented in the following equation:
467
468 $$Y_{ij} = \mu + b_i + \beta_1 x_{1j} + \beta_2 x_{2j} + \beta_3 x_{3j} + \beta_4 x_{4j} + \varepsilon \qquad (2)$$
469
470 Where Y is the furan concentration, μ the intercept, b the effect of vegetable type, β_1, β_2 and β_3 the
471 effects of fructose, glucose and sucrose concentration and β_4 the effect of degraded vitamin C for the i^{th}
472 vegetable purée and the j^{th} repetition of the treatment. The results of the statistical analysis for the fixed
473 factor effects are given in **Table 3**.
474

The results of the statistical analysis clearly demonstrate that there were significant correlations between both the amounts of free sugars and degraded vitamin C and the furan concentration of the thermally-treated purées. For glucose, sucrose and vitamin C, these correlations were positive, as expected, based on literature findings. For fructose, the correlation was negative. This contradicts previously reported findings by Limacher *et al.* (2008), who stated that pentose sugars tend to generate more furan than hexoses. It should be stressed however that the mixed model regression was only a tool, able to identify correlations between certain parameters, but not causal relationships. Instead of a real inverse relationship between initial fructose concentration and furan formation, it is more likely that furan formation in the vegetable purées was influenced by interactions with other precursors or reaction conditions. The associations between the different precursors and furan concentration can be compared with each other by looking at the *Pr*-values for each correlation. The strongest association was observed for vitamin C, followed by fructose, sucrose and glucose, in that order. This agreed with the literature, where many studies have identified vitamin C as the most efficient furan precursor in model systems (Locas & Yaylayan, 2004; Mark *et al*., 2006; Fan *et al*., 2008; Limacher *et al*., 2008; Owczarek-Fendor *et al*., 2012). The importance of free sugars could be attributed to their high concentrations in foods like fruit- and vegetable-based products. However not varied in this study, furan formation seems to be strongly influenced by reaction conditions such as pH and redox condition (Mark *et al*., 2006; Limacher *et al*., 2007; Fan *et al*., 2008; Limacher *et al*., 2008; Owczarek-Fendor *et al*., 2010a; Huang *et al*., 2011; Owczarek-Fendor *et al*., 2012). In low-acid products, furan formation from carbohydrates is reported to be slightly favored compared with furan formation from ascorbic acid. For both precursors, more furan seems to be formed when the oxygen concentration is increased. In any case, literature data should be interpreted with prudence, given the complexity of the combined effects of reaction conditions and interactions.

3.3.2. Total carotenoids and free amino acids as minor furan precursors

In addition to carbohydrates, ascorbic acid and unsaturated fatty acids, carotenoids and certain amino acids have been reported as a precursor for the formation of furan. Little is known about their potency or degradation mechanisms, but it seems that they are only minor precursors (Fan, 2005; Crews & Castle, 2007; Moro *et al*., 2012). In an attempt to learn more about the importance of these other intrinsic properties for furan formation in vegetable-based food systems, the statistical analysis of Section 3.3.1 was extended with both precursors added to the mixed model.

3.3.2.1. Characterization of the starting material

The concentration of total carotenoids in the blanched vegetable purées was determined spectrophotometrically according to the procedure of Knockaert *et al.* (2011). Orange carrot had the highest concentration of total carotenoids (9800 µg/100 g purée), followed by spinach, red carrot and pumpkin. Smaller amounts of total carotenoids (600-1100 µg/g purée) were found in bell pepper, broccoli and the other carrot varieties.

Free amino acids were analyzed using the EZ:faast amino acid analysis kit (Phenomenex, USA). For each vegetable, an estimation of the total amount of free amino acids was made by finding the sum of the response ratios of each individual amino acid and an internal standard (norvaline). High amounts of free amino acids were detected in broccoli (estimated value of 38) and potato (estimated value of 35). The amounts of free amino acids in the other vegetable purées were very comparable to each other (estimated values between 5 and 12).

3.3.2.2. Mixed model regression

The results of the statistical analysis for the fixed factor effects are shown in **Table 4**. The addition of two fixed factors, total carotenoids and free amino acids, to the mixed model resulted in slightly different results compared with the original model set up in Section 3.3.1. The significant correlations between the amounts of fructose and degraded vitamin C and the furan concentration of the thermally-treated purées remained, but for glucose and sucrose, the correlations were no longer significant. Apart

526 from that, the addition of total carotenoids and free amino acids did not seem to add a lot of extra
527 information to the model. The *Pr*-values of both precursors were far from significant and their
528 parameter estimates were close to zero. These results agreed with our expectations that carotenoids and
529 amino acids were only minor precursors for furan formation compared with carbohydrates and vitamin
530 C. The structure of carotenoids is similar to that of unsaturated fatty acids, so it can be assumed that
531 their degradation reactions follow similar pathways. This would imply that furan is mainly formed at
532 unrealistically high levels of carotenoid oxidation, which might explain why total carotenoids did not
533 show up as an important furan precursor in the mixed model regression. However, carotenoids could
534 still play an important role in furan formation because of their effects on the redox condition of the
535 system. Amino acids for their part, can produce furan when two of their degradation products,
536 acetaldehyde and glycolaldehyde, react together in an aldol condensation reaction (Locas & Yaylayan,
537 2004). Most amino acids are only able to generate acetaldehyde and therefore they need the presence of
538 another compound (such as reducing sugars) to form furan. Given the reactivity of amino acids with
539 other compounds, it could be expected that the formation of furan directly from amino acids was rather
540 limited. Indirectly, amino acids could have a major influence when interactions with other compounds
541 are taking away or providing building blocks for furan formation. For both precursors however, it
542 seemed that the effects on the furan formation in vegetable-based food products were limited, even
543 indirectly. Moreover, it should be noted that the concentrations of carotenoids and free amino acids
544 were only small compared with the concentrations of major precursors such as carbohydrates and
545 vitamin C.
546
547 *3.4. Methylfuran formation in vegetable purées*
548 In addition to furan, the treated vegetable purées were analyzed for 2- and 3-methylfuran. As
549 mentioned in the introduction, both these alkylated derivatives of furan may be important from a safety
550 point-of-view, since they are likely to undergo the same metabolic fate as furan.
551

3.4.1. Effect of different sterilization techniques on the methylfuran formation in vegetable purées

In a similar approach to that for furan, the integrated impact of conventional thermal processing and innovative HPHT processing on the formation of 2- and 3-methylfuran was compared. For both methylfurans, this was the first time that the effect of HPHT processing had been investigated in a targeted manner. The concentrations of 2- and 3-methylfuran in the HPHT- and thermally-treated vegetable purées, shown in **Fig. 5**, were determined in one analytical run, together with the analysis of furan.

In most vegetables, the concentrations of both methylfurans were very similar to each other. When considered individually, the concentrations were slightly lower compared with the corresponding concentration of furan (*cf.* **Fig. 2**), but together, for most vegetables they could not be ignored. A clear reduction in the total amount of methylfuran could be observed for the HPHT-treated purées, where concentrations dropped to levels close to the analytical limits (1-2 ng/g purée). A mixed model regression (**Table 5**, fixed effects), analogous to the procedure applied in Section 3.2, confirmed that the reduction in the total amount of methylfuran for the HPHT-treated vegetable purées was significant. Similarly to the reduction of furan observed earlier, the reduction of methylfuran could be explained by the effect of high pressure on the rate constants of the chemical reactions, by the different temperature-time profiles of the treatments or by a combination of both effects. To obtain a clear insight into the individual effect of the processing variables, again, a kinetic study needs to be set up.

HPHT processing has been proved again to show great potential in mitigating concentrations of furan-like compounds in conduction-heated vegetable-based food products. If the health concerns about furan and both methylfurans are justified, HPHT processing could be a very interesting alternative for simultaneously reducing both these compounds in sterilized vegetable-based products. The reduction of methylfuran by HPHT treatment agreed with recent findings by Kebede *et al.* (2013) (3-methylfuran in bell pepper and 2-methylfuran in spinach) and Vervoort *et al.* (2013) (3-methylfuran in carrot), who have observed the same trend when applying an untargeted analytical approach to compare the process impact of conventional thermal and HPHT processing on different vegetable-based food systems.

578

579 *3.4.2. Effect of chemical composition on the methylfuran formation in thermally-treated vegetable*
580 *purées*

581 The highest amounts of methylfuran (2- and 3-methylfuran added together) were found in the
582 thermally-treated purées (**Fig. 5**). A large variation could be observed between bell pepper (23 ng/g
583 purée), broccoli (16 ng/g purée), red carrot (11 ng/g purée), pumpkin (10 ng/g purée) and spinach (9
584 ng/g purée), which seemed to be very susceptible to the formation of methylfuran, and potato, red beet
585 and the other carrots, where almost no methylfuran was formed. For red beet, the lack of methylfuran
586 formation was remarkable, because it had the highest furan concentration of all vegetables.

587 Vegetable matrices where the formation of high amounts of methylfuran is combined with high
588 amounts of furan, might be identified as priority matrices with regard to (methyl)furan mitigation. In
589 this context, it should be noted that 2- and 3-methylfuran have not yet officially received the same
590 toxicological classification as furan. The simultaneous reduction of furan and methylfuran in
591 conventional thermally processed foods could be challenging, because the reaction pathways leading to
592 the formation of both alkylated furans might be different from furan. There is a need for more insight
593 into the mechanisms of methylfuran formation during thermal processing and therefore the statistical
594 approach from Section 3.3 was repeated for the total amount of methylfuran found in the thermally-
595 treated purées. Since all the food components characterized in the context of furan formation (free
596 sugars, vitamin C, total carotenoids and free amino acids) are also named as precursors for the formation
597 of methylfuran, the concentrations resulting from the characterization of the blanched vegetable purées
598 in Sections 3.3.1.1 and 3.3.2.1 were adopted for the mixed model regression of methylfuran in the
599 thermally-treated purées.

600 The discussion of this model is limited to the results of the model containing all precursors, since the
601 results of the basic model containing only free sugars and vitamin C were very comparable. From the
602 results, shown in **Table 6**, only one significant correlation could be observed, that is between the
603 amount of degraded vitamin C and the total amount of methylfuran detected in the thermally-treated

purées. Weak correlations (but not significant) were present for sucrose and total carotenoids. Fructose, glucose and free amino acids were not correlated with the total amount of methylfuran. In the literature, only small amounts of methylfuran are found in model systems composed of sugars, ascorbic acid or unsaturated fatty acids (Becalski & Seaman, 2005; Mark *et al*., 2006; Limacher *et al*., 2007; Limacher *et al*., 2008). The formation of methylfuran is however strongly increased in binary mixtures, especially in the presence of amino acids. It is assumed that the formation of methylfuran involves more fragmentation and recombination steps than for the formation of furan. The highest concentrations of methylfuran are found in Maillard systems containing mixtures of sugars and amino acids, as opposed to systems containing ascorbic acid and amino acids, in which the amounts of methylfuran remain rather low. In this respect, it might seem surprising that, in the present study, vitamin C was the only precursor showing a significant correlation with the total amount of methylfuran. It is possible that the fragmentation of ascorbic acid was further enhanced in real systems such as vegetable-based systems because of the presence of a wide variety of natural compounds. Furthermore, the formation of methylfuran has been described as being enhanced in aqueous solutions and as a function of pH, conditions at which the fragmentation of compounds is favored (Limacher *et al*., 2007). This might explain why some of the vegetable purées in this study were particularly vulnerable to methylfuran formation.

4. Conclusions

Following recommendations of the Joint FAO/WHO Codex Committee on Contaminants in Foods (2011), the present study addressed the need for furan reducing measures at the level of food production. For the first time in the literature, the integrated impact of HPHT processing on the formation of furan has been investigated and compared with the impact of conventional thermal processing. A very interesting finding was the clearly different and lower concentrations of furan found in a wide range of individual vegetable purées after HPHT treatment. The furan concentrations dropped to levels close to the analytical limits (1-2 ng/g purée), which means that almost no furan was detected in the HPHT-

630 treated purées, even though they were treated under industrially relevant conditions for sterilization
631 (process value F_0 = 5 min). The furan reduction could be explained by the effect of high pressure on the
632 rate constants of the chemical reactions, by the different temperature-time profiles of the treatments or
633 by a combination of both effects. The highest amounts of furan were found in the thermally-treated
634 purées. Red beet (15 ng/g purée), bell pepper (11 ng/g purée) and spinach (10 ng/g purée) were
635 classified as possible risk matrices for the formation of furan. Using a mixed model regression, vitamin
636 C and free sugars could be identified as the main precursors leading to the formation of furan. Vitamin
637 C is believed to be an efficient precursor, whereas the importance of sugars seems to follow from their
638 high concentrations in fruit- and vegetable-based products. Next to furan, the HPHT- and thermally-
639 treated vegetable purées were analyzed for 2- and 3-methylfuran, in view of their possible toxicological
640 relevance to the risk evaluation of furan. For most of the vegetables tested and bell pepper in particular,
641 the total amount of methylfuran found in the thermally-treated purées (9-23 ng/g purée) could not be
642 ignored. Interestingly, a clear reduction of the concentrations could be observed for the HPHT-treated
643 vegetable purées, analogous to furan.

644 The choice for real systems, processed at an industrially relevant process value for sterilization, results
645 in relevant furan concentrations and conclusions from the application point-of-view. HPHT processing
646 presents itself as a really interesting alternative for furan reduction in sterilized vegetable-based systems,
647 but the mechanisms behind the reduction are not fully understood. To obtain a clear insight into the
648 individual effect of the processing variables, furan formation should be studied over a range of pressure,
649 temperature and time conditions. Furthermore, it would be interesting to evaluate the potential of HPHT
650 processing with respect to the reduction of other process-induced contaminants. At this moment, the
651 specific applications of HPHT processing are still under research and equipment and operating costs
652 might limit its use to high-value added foods. For many conduction-heated products, conventional
653 thermal processing will remain the standard sterilization technique. A logical mitigation strategy in
654 those products seems to be intervention in the reaction mechanisms leading to furan formation. Vitamin
655 C and free sugars were identified as the main precursors for furan formation, but the complexity of the

reaction network and mutual interactions might hinder an easy solution for furan reduction. In order to fully understand the relationship between the degradation of both precursors and furan, the reactions should be further investigated and evoked in real food systems, under closely controlled conditions. The same goes for methylfuran, where only the first steps were made towards product optimization, just like for furan. Vegetable matrices, where the formation of high amounts of methylfuran is combined with high amounts of furan, might be identified as priority matrices with regard to furan reduction. In anticipation of more information on the toxicological effects and the reaction pathways leading to the formation of both methylfurans, it would therefore be relevant to continue the monitoring of both compounds. As shown in the present study, the methylfuran concentrations can easily be estimated in one analytical run together with furan, with a minimum of modifications.

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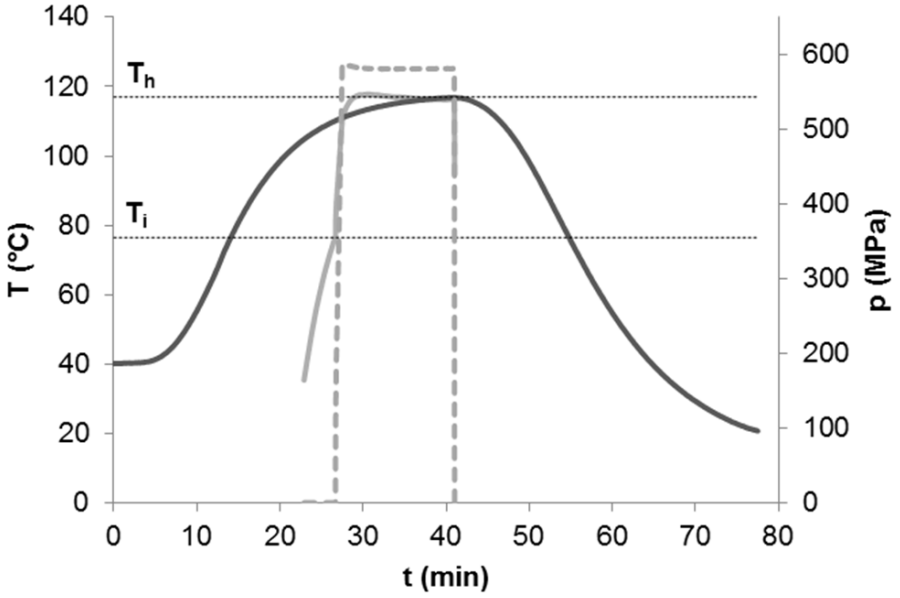

Fig. 1. The profiles of thermal treatment with product temperature (bold dark line) and HPHT treatment with product temperature (bold light line) and pressure (dashed light line). For the HPHT treatment, sequential steps were followed: equilibration of the pressure vessel to the holding temperature, T_h; insertion of the sample in the isolated sample holder in the vessel; preheating of the sample to initial temperature, T_i (experimentally determined and dependent on pressure and required T_h); fast pressure build-up accompanied by compression heating; the sample temperature is allowed to equilibrate to T_h during 1 min isolation time; the process pressure was maintained during a holding time; the pressure is released, which is associated with a fast temperature drop.

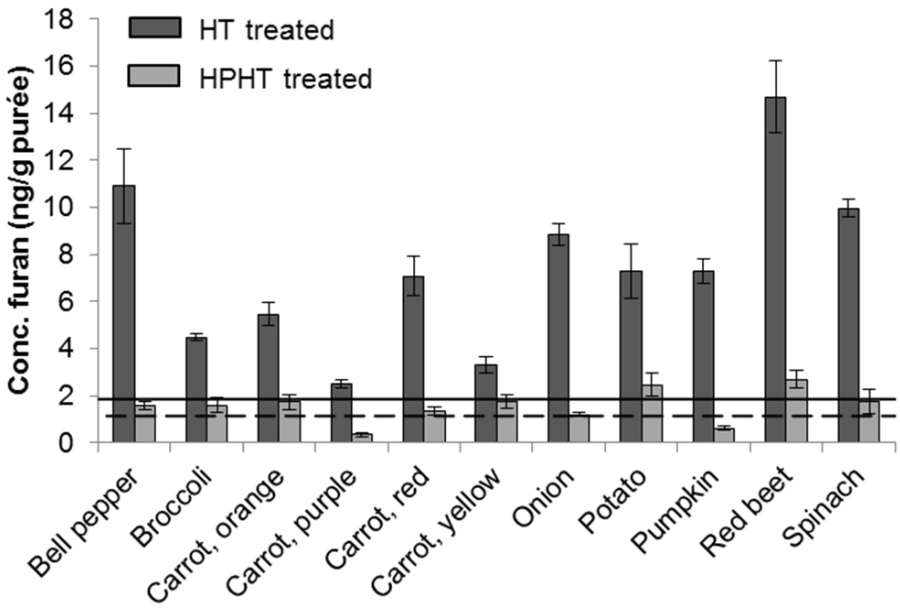

Fig. 2. Furan concentration of the vegetable purées sterilized under thermal and HPHT conditions, with indication of the decision limit (dashed line) and the detection capability (solid line).

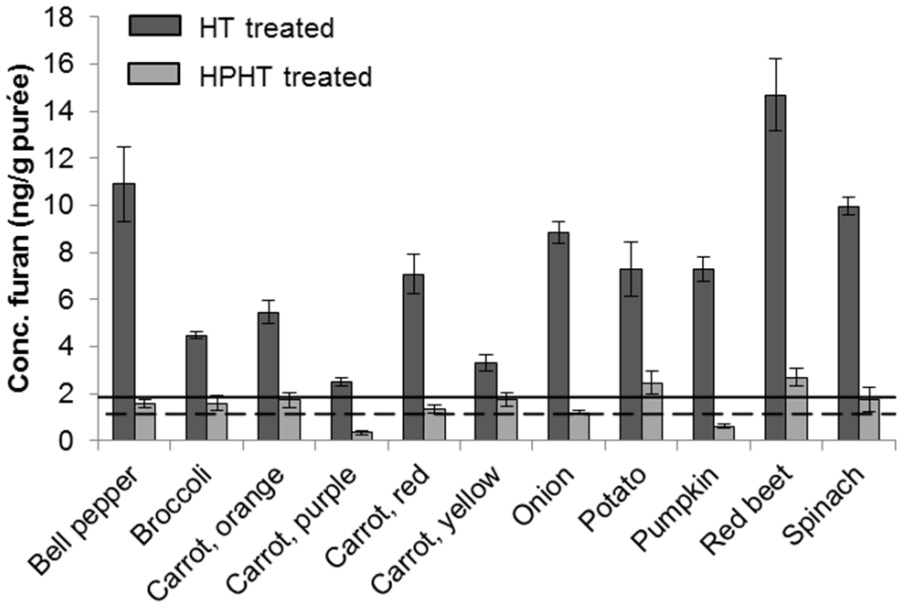

Fig. 3. Free sugar content of the blanched vegetable purées. Only fructose, glucose and sucrose were detected in quantifiable amounts.

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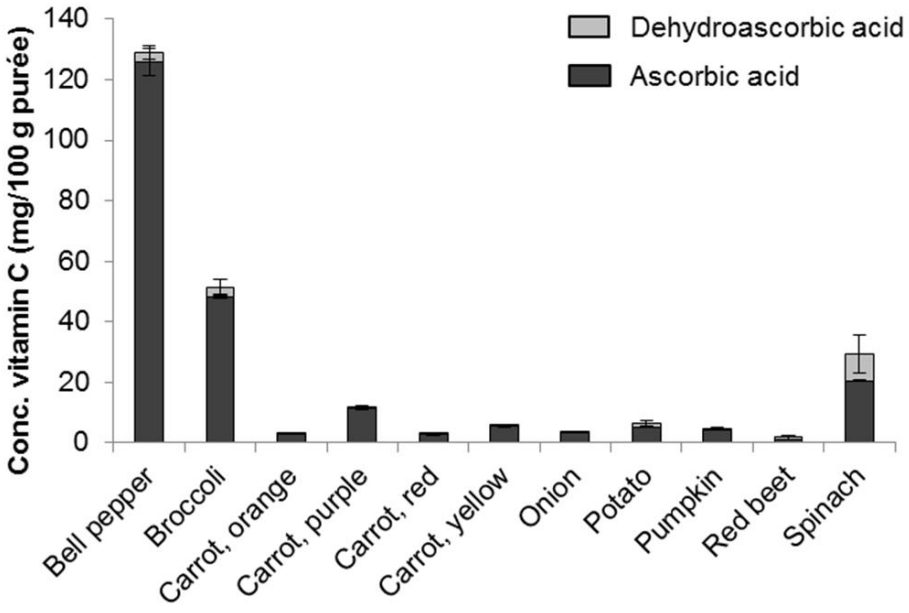

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841 **Fig. 4.** Vitamin C content of the blanched vegetable purées. Both the amount of ascorbic acid and

842 dehydroascorbic acid are represented.

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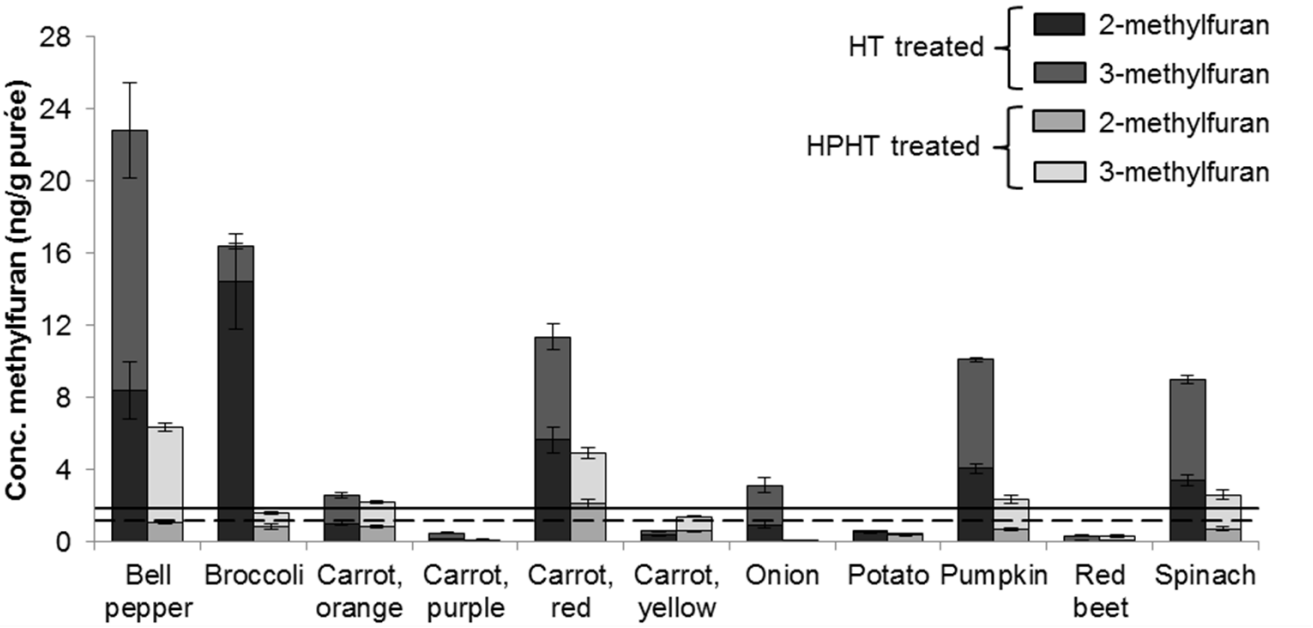

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846 **Fig. 5.** Concentrations of 2-methylfuran and 3-methylfuran in the vegetable purées treated at thermal
847 conditions and in the purées treated at HPHT conditions, with indication of the decision limit (dashed
848 line) and the detection capability (solid line).

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850 **List of tables**

851

852 **Table 1.** Basic characteristics of the selected vegetables.

Vegetable	Cultivar/variety	Botanical family	Plant part	Colour
Bell pepper	California Wonder	Solanaceae	Fruit	Green
Broccoli	Italica	Brassicaceae	Flower	Green
Carrot				
- Orange	Mourac	Apiaceae	Root	Orange
- Purple	Deep Purple	Apiaceae	Root	Purple
- Red	(Unknown)	Apiaceae	Root	Red
- Yellow	Mellow Yellow	Apiaceae	Root	Yellow
Onion	Sunskin	Alliaceae	Bulb	White
Potato	Bintje	Solanaceae	Tuber	White
Pumpkin	Mucha	Cucurbitaceae	Fruit	Orange
Red beet	Rubra	Chenopodiaceae	Root	Red
Spinach	Falcon	Chenopodiaceae	Leaf	Green

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856 **Table 2.** Statistical output of the selected mixed model to obtain insight into the effect of sterilization

857 technique on furan formation in vegetable purées: solutions for fixed and random effects ($\alpha = 0.05$).

Fixed effects	**Treatment type**	**Estimate**	**Standard error**	**t-Value**	**Pr > |t|**
Intercept (μ)	Thermal	7.4490	0.6297	11.83	< 0.0001
Treatment (β)	HPHT	–5.8958	0.3870	–15.24	< 0.0001
Random effects	**Vegetable type**	**Estimate**	**Standard error**	**t-Value**	**Pr > |t|**
Vegetable (b_i)	Bell pepper	1.5731	N/A	N/A	N/A
	Broccoli	–1.3045	N/A	N/A	N/A
	Carrot, orange	–0.8028	N/A	N/A	N/A
	Carrot, purple	–2.7537	N/A	N/A	N/A
	Carrot, red	–0.2502	N/A	N/A	N/A
	Carrot, yellow	–1.7533	N/A	N/A	N/A
	Onion	0.4851	N/A	N/A	N/A
	Potato	0.2954	N/A	N/A	N/A
	Pumpkin	–0.4771	N/A	N/A	N/A
	Red beet	3.7641	N/A	N/A	N/A
	Spinach	1.2239	N/A	N/A	N/A

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Table 3. Statistical output of the selected mixed model to obtain insight into the effect of chemical composition on furan formation in thermally-treated vegetable purées: estimated fixed effects of fructose, glucose, sucrose and vitamin C ($\alpha = 0.05$).

Fixed effect	**Estimate**	**Standard error**	**t-Value**	**Pr > |t|**
Intercept (μ)	5.5930	2.1426	2.61	0.0349
Fructose (β_1)	–42.1907	14.2585	–2.96	0.0046
Glucose (β_2)	20.8758	9.9266	2.10	0.0401
Sucrose (β_3)	5.1810	2.1890	2.37	0.0216
Vitamin C (β_4)	0.1851	0.0360	5.15	< 0.0001

866

867 **Table 4.** Statistical output of the selected mixed model to obtain insight into the effect of chemical
868 composition on furan formation in thermally-treated vegetable purées: estimated fixed effects of
869 fructose, glucose, sucrose, vitamin C, total carotenoids and total free amino acids ($\alpha = 0.05$).

Fixed effect	**Estimate**	**Standard error**	**t-Value**	**Pr > |t|**
Intercept (μ)	8.0782	5.0113	1.61	0.1679
Fructose (β_1)	–41.7867	16.9962	–2.46	0.0172
Glucose (β_2)	18.6565	12.9291	1.44	0.1548
Sucrose (β_3)	4.2108	2.9606	1.42	0.1607
Vitamin C (β_4)	0.1882	0.0381	4.95	< 0.0001
Total carotenoids (β_5)	–0.0014	0.0318	–0.05	0.9640
Total free amino acids (β_6)	–0.0498	0.07883	–0.63	0.5302

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873 **Table 5.** Statistical output of the selected mixed model to obtain insight into the effect of sterilization
874 technique on methylfuran formation in vegetable purées: solutions for fixed and random effects (α =
875 0.05).

Fixed effect	**Treatment type**	**Estimate**	**Standard error**	**t-Value**	**Pr > |t|**
Intercept (μ)	Thermal	6.0175	1.3685	4.40	0.0013
Treatment (β)	HPHT	–4.0246	0.6063	–6.64	< 0.0001
Random effect	**Vegetable type**	**Estimate**	**Standard error**	**t-Value**	**Pr > |t|**
Vegetable (b_i)	Bell pepper	10.0386	N/A	N/A	N/A
	Broccoli	–0.5068	N/A	N/A	N/A
	Carrot, orange	–1.5560	N/A	N/A	N/A
	Carrot, purple	–3.5254	N/A	N/A	N/A
	Carrot, red	3.9246	N/A	N/A	N/A
	Carrot, yellow	–2.8575	N/A	N/A	N/A
	Onion	–2.2784	N/A	N/A	N/A
	Potato	–3.4931	N/A	N/A	N/A
	Pumpkin	2.1227	N/A	N/A	N/A
	Red beet	–3.5126	N/A	N/A	N/A
	Spinach	1.6441	N/A	N/A	N/A

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879 **Table 6.** Statistical output of the selected mixed model to obtain insight into the effect of chemical
880 composition on methylfuran formation in thermally-treated vegetable purées: estimated fixed effects of
881 fructose, glucose, sucrose, vitamin C, total carotenoids and total free amino acids ($\alpha = 0.05$).

Fixed effect	**Estimate**	**Standard error**	**t-Value**	**Pr > |t|**
Intercept (μ)	–2.0696	6.4933	–0.32	0.7628
Fructose (β_1)	–3.4551	22.0228	–0.16	0.8759
Glucose (β_2)	5.2593	16.7518	0.31	0.7548
Sucrose (β_3)	6.0086	3.8370	1.57	0.1232
Vitamin C (β_4)	0.4478	0.0498	8.99	< 0.0001
Total carotenoids (β_5)	0.0534	0.0412	1.29	0.2009
Total free amino acids (β_6)	–0.0737	0.1021	–0.72	0.4733

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